A simple, inexpensive thin-layer chromatography method for the analysis of theophylline tablets*

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A simple, low-cost thin-layer chromatography (TLC) procedure to estimate the quality of simple pharmaceuticals in tablet form is described together with easily built equipment to carry out the test in the field. The approach is demonstrated for theophylline, but can be used to assay the drug content of any tablet or to determine its dissolution or disintegration characteristics. The procedure can be used in the field without the need for any instrumentation.

In many areas of the world, there is a need to estimate the quality of drug preparations using a minimum of laboratory resources. An acceptable screening technique would rapidly and inexpensively establish the level of a specified drug (including its absence), indicate the disintegration characteristics, and allow the detection of significant quantities of foreign substances. If such a screening method were available, health care professionals could rapidly evaluate the quality of drug products and determine whether further, more sophisticated testing were warranted.

The thin-layer chromatography (TLC) method we describe here allows a person of reasonable manipulative skills, but lacking extensive technical training or laboratory resources, to perform screening examinations successfully on drug formulations. Furthermore, it is suitable for use in the field.

The method is inexpensive and rapid and, because it involves comparative testing using a reference product, does not require the use of sophisticated apparatus. It is intended that the procedure be used to determine whether a product distinctly passes or fails any of a number of test attributes. If a product fails a given attribute, confirmatory testing using the appropriate legally prescribed methods would be required before taking regulatory action.

Method

Apparatus and reagents

The apparatus and reagents described below are required to carry out the method.

- *Standard tablet.* Theophylline tablets (200-mg label declaration) packaged in a Strip-Pak® (Slo-Phyllin®, control number: 67247).[a]
- *Items 1A and 1B.* A plastic bag (28-cm long) made out of 5-cm wide, 0.006-gauge polyethylene tubing.[b] Make the bag by sealing one end three times across the tubing (at 3 mm, 6 mm, and 9 mm, respectively, from the end) using a 20-cm Impulse Sealer®.[c]
- *Item 2.* A plastic Tube-Pak® (size 32D, 5-cm internal diameter, 6-cm outer diameter, 3-mm wall thickness, 28-cm long).[c] Round off the inside edges.
- *Items 3A and 3B.* A 120-ml (4.5-ounce) plastic specimen container (item 3A) with a plastic screw cap (item 3B).[d]
- *Item 4.* Two 5-cm long plastic binding slides cut from a 28-cm (11-inch) binding slide.[e]
- *Item 5.* Two metal paper clamps approximately 3-cm wide.[e]
- *Items 6A and 6B.* A glass Pasteur-type pipette (15-cm long) (item 6A) and a 2-ml rubber dropper bulb (item 6B).[f] Item 6 is the combination of items 6A and 6B. A total of five droppers and bulbs are required.
- *Items 7A, 7B, and 7C.* Three disposable glass vials (8-mm diameter, 30-mm deep) with plastic caps.[g]
- *Items 8A and 8B.* A disposable 5-µl microcapillary (item 8A) with microcapillary holder (item 8B).[h]

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[a] From William H. Rorer Inc., Fort Washington, PA 19034, USA.

[b] From Packaging Aids Corporation, San Fransisco, CA 94107, USA.

[c] From Consolidated Plastics Company, Twinsburg, OH 44087, USA.

[d] From Superior Plastic Products, Cumberland, RI 02864, USA.

[e] Model No. 155. From Ribbons and Rolls Business Center, Collinsville, IL 62234, USA.

[f] From Fisher Scientific International, Springfield, NJ 07081, USA.

[g] From Sun Brokers Inc., Wilmington, NC 28402, USA.

[h] From Drummond Scientific Co., Broomall, PA 19008, USA.

• *Item 9.* A TLC plate.[i] Cut a 5-cm × 10-cm rectangle from a 20-cm × 20-cm aluminium TLC plate that has been precoated with a 0.2-mm layer of silica gel 60 F-254. Make an aluminium border around this rectangle by removing a 2-mm band of silica gel from both 10-cm edges and one 5-cm edge. Without damaging the silica gel layer, use a pencil to make two small dots 2.5 cm from the bottom (the edge without a border) and 1.5 cm from each edge; label these 7A (left side) and 7C (right side). Make another dot at the same level midway between the dots 7A and 7C and label this 7B. Finally, draw a line 6 cm above and parallel to that made by the dots.

• *Item 10.* Developing solvent (4.7 ml). This consists of glass-distilled chloroform and acetone (1 : 1 v/v) in a completely filled glass vial sealed with an aluminium foil-lined screw cap.[j] The vial threads should be wrapped with Teflon® tape before capping to reduce loss by evaporation.

• *Items 11A and 11B.* Two saturators, 7.7-cm × 10-cm (item 11A) and 5-cm × 10-cm (item 11B), cut from a 20-cm square sheet of heavy-duty Whatman No. 3 filter-paper (0·3-mm thick).

• *Item 11C.* A TLC plate support (Fig. 1), prepared by cutting a 11.8-cm × 7-cm rectangle from a sheet of 0.3-mm thick aluminium. Cut 2-cm isosceles triangles off both corners on one end (bottom) and 1-cm squares from both corners on the other end (top) of the piece. Round off all the corners and smooth all the edges on the remaining piece. To form the side ridges, fold the metal 5 mm from the edge to make a right angle along each long side. Form the bottom ridge by making a 170°-bend 5 mm from the bottom edge, and the top ridge by introducing a 170°-bend 1 cm from the top edge. All bends are made in the same sense. The plate support should be sufficiently long to prevent the heavy-duty filter-paper and the TLC plate from bending when placed in it.

• *Item 12.* A polyethylene development bag (20-cm long) made from 8-cm wide, 0.006-gauge polyethylene tubing.[k] Using the 20-cm Impulse Sealer®, make the bag by sealing three times across the tubing (at 3 mm, 6 mm and 9 mm from one end).

• *Item 13.* A two-component visualizing agent (*l*) made up as follows: solution A—completely dissolve 8 g of potassium iodide in 200 ml of 95% (v/v) ethanol and then dissolve 32 g of iodine in this solution; solution B—carefully mix 25 ml of concentrated hydrochloric acid with 75 ml of distilled water and to this add 100 ml of 95% (v/v) ethanol and stir the mixture. Solution C—mix solution A and solution B to obtain the visualizing agent. Put 4-ml aliquots of the visualizing agent in 4-ml screw-capped glass vials equipped with Teflon® seals (PTFE SEPTUM®) seals.[l]

• *Item 14.* A polyethylene visualization development bag (13-cm long) made from 8-cm wide, 0.006-gauge polyethylene tubing.[m] To make the bag, seal three times across the tubing (3 mm, 6 mm, and 9 mm from one end) using a 20-cm Impulse Sealer®. Form a flap across the top of the bag by cutting out an 8-cm × 2.5-cm rectangle from one side of the open end. Finally, make the bag 6-cm wide by forming a seal about 1 cm from the edge of each side along its entire length.

Tablet disintegration

• *Step 1.* Before proceeding, place items 1A to 14 in numerical order on the work surface and read all the instructions.

• *Step 2.* Carefully insert as far as possible the closed ends of the polyethylene tubes (items 1A and 1B) into the plastic Tube-Pak® (item 2), taking care that the tubes are not bent or crimped. The closed ends of the polyethylene tubes should be at the bottom of item 2. Fit the bottom of the latter snugly in place into the top side of the screw cap (item 3B).

• *Step 3.* Using the specimen container (item 3A), pour 100 ml of distilled water into item 1A. Similarly, pour distilled water (100 ml for 200–300-mg theophylline tablets; 50 ml for 100-mg or 125-mg tablets) into item 1B.

• *Step 4.* Drop a standard theophylline tablet into item 1A and a sample tablet into item 1B; immediately start timing using a clock or by counting 1, and 2, and 3, etc. Steps 5–10 should be performed as quickly as possible after adding the tablets, making sure to keep counting or timing.

• *Step 5.* Close the bag (item 1A) that contains the standard tablet by making three 1-cm folds across the top. *Note.* For 100-mg and 125-mg tablets only, the first fold should be made 13 cm and the second and third folds 1 cm each from the top.

• *Step 6.* Seal the folds in place by sliding a plastic binder (item 4) over them.

• *Step 7.* Fix the binder in place using a metal paper clamp (item 5), and flip the wires on the clamp down over the closed bag containing the standard tablet (item 1A). The other bag containing the sample tablet is closed in a similar manner (item 1B). Both bags are then suspended with their folded edges resting on the upper edges of the plastic tube (item 2) (Fig. 2), and the specimen container (item 3A) is fitted snugly over the top of the tube (Fig. 3).

[i] From E.M. Laboratories Inc., Elmsford, NY 10523, USA.

[j] See footnote *g*, p. 555.

[k] See footnote *b*, p. 555.

[l] See footnote *g*, p. 555.

[m] See footnote *b*, p. 555.

Fig. 1. **Pattern for making the TLC plate support (item 11C).**

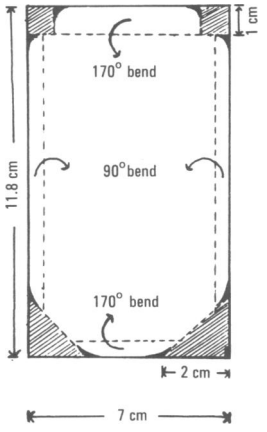


Fig. 2. **The polyethylene bags (items 1A and 1B) held in place by the plastic binding slide (item 4) and the metal paper clamps (item 5).**

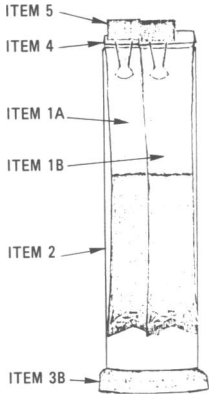


Fig. 3. **The complete assembly fitted with the specimen container (item 3A), ready to be shaken.**

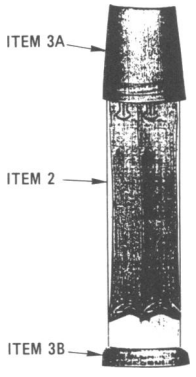


Fig. 4. **Illustration of how a sample should be removed from the polyethylene bag after the tablets have disintegrated.**

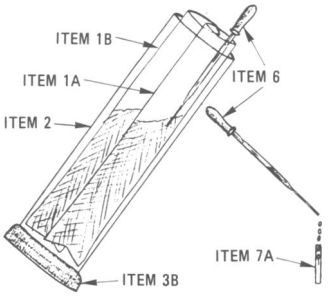


Fig. 5. **Illustration of how to spot the TLC plate (item 9) with the capillary tube (item 8A).**

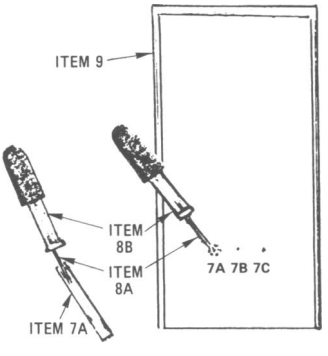


Fig. 6. **The spotted TLC plate and the saturator (item 11B) positioned in the metal plate support (item 11C).**

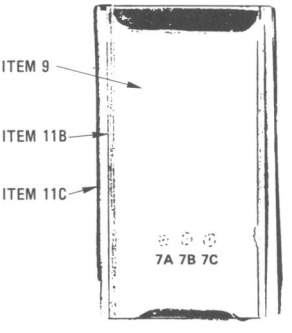


Fig. 7. **Pouring the developing solvent (item 10) into the polyethylene development bag (item 12). Item 11A is the saturator.**

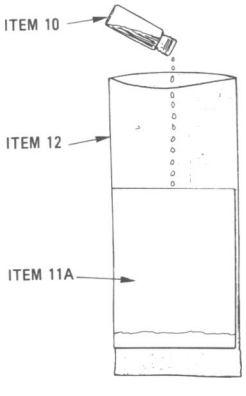


Fig. 8. **The TLC plate developing in the closed polyethylene development bag.**

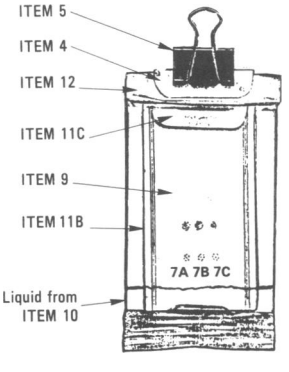
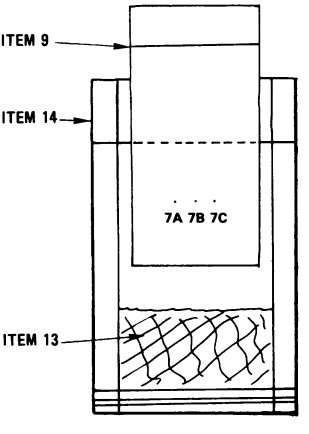


Fig. 9. **Placing the developed and dried TLC plate in the visualization development bag (item 14).**



• *Step 8.* Hold the entire assembly horizontally and shake it vigorously, stopping at intervals only long enough to observe the condition of the tablets.
• *Step 9.* Note the times taken for the sample tablet and the standard tablet to disintegrate completely and stop shaking the tube when both tablets have disintegrated.
• *Step 10.* Set the plastic tube assembly on a level surface.

Qualitative and quantitative analyses

• *Step 11.* Remove the plastic specimen container (item 3A) and open the polyethylene bags (items 1A and 1B) by carefully taking off the clamps and binders (items 4 and 5). Be very careful not to agitate or spill the solutions.
• *Step 12.* Using a clean glass dropper (item 6A) with a rubber bulb (item 6B), transfer about 15 drops of liquid from the bag that contains the standard tablet (item 1A) to a glass vial (item 7A). It may be necessary to tilt the entire assembly to reach the solution (Fig. 4). To dilute the sample solution, use another clean glass dropper to transfer the appropriate number of drops of liquid (see Table 1) from the sample tablet bag (item 1B) to a second glass vial (item 7B). By means of a further clean glass dropper then add the appropriate number of drops of distilled water (Table 1) to item 7B. Cover both glass vials with plastic caps and shake the sample vial vigorously 10 or 12 times.
• *Step 13.* Using a clean glass dropper for each liquid, transfer 6 drops of the solution of the standard tablet from its vial and 4 drops of distilled water to a third glass vial (item 7C), cover it with a plastic cap, and shake it vigorously 10 or 12 times.
• *Step 14.* Hold a capillary tube (item 8A) between the fingertips or insert it into the white or yellow end of a capillary holder (item 8B) and touch the capillary tip on the surface of the liquid in item 7A—the glass vial that contains the standard tablet solution.

Table 1: **Instructions for diluting the liquid from the sample tube (item 1B)**

Weight of sample tablet (mg)	No. of drops of:	
	Solution from item 1B	Distilled water
100	10	0
125	8	2
200	10	0
225	8	1
250	8	2
300	8	4

It may be necessary to tilt the vial to do this. Keep the capillary in the liquid until it is filled, and then gently hold its tip to the pencil-mark dot labelled 7A on the TLC plate (Fig. 5). The liquid should completely transfer to the white surface of the TLC plate—this occurs best if the tube is held perpendicular to the surface of the plate. If the liquid does not transfer, place a fingertip over the hole in the black top of the capillary holder and squeeze it. Using clean capillary tubes for each application, repeat step 14 to apply the sample solution from its vial to the spot labelled 7B and the solution from vial 7C to the spot marked 7C. The spotted end of the TLC plate is the bottom.
• *Step 15.* Let the spots dry (approximately 5 minutes). In the meantime, lay the metal surface of the TLC plate on the smaller saturator (item 11B). Touch the white silica gel layer on the plate as little as possible since the presence of fingerprints might affect the results of the test. With the silica gel layer of the plate facing towards the observer, insert both the TLC plate and the saturator into the plate support (item 11C) with the spotted end in the narrow fold and the plain end in the wider fold (Fig. 6).
• *Step 16.* Place the larger saturator (item 11A) in the polyethylene development bag (item 12) and pour the contents of one vial of developing solvent (item 10) into the bag, ensuring that the saturator is completely wetted (Fig. 7). *Avoid getting the developing solvent on the skin, clothing, or in the eyes.* Carefully insert the assembly from step 15 (items 9, 11B, and 11C) into the upright and level development bag, ensuring that the solvent is not agitated during this process. The silica gel layer of the plate should face towards the observer. The bottom of the bag and the level of the solvent must always be horizontal. Close the bag as noted in steps 5, 6, and 7. Suspend the entire assembly using the wires on the paper clamp (item 5), or support it by some other means, so that it is kept vertical while the bottom is kept horizontal. The solvent must rise evenly (Fig. 8).
• *Step 17.* Remove the TLC plate immediately after the solvent band reaches the pencil-mark line and thoroughly air dry it for about an hour.
• *Step 18. Complete this step as quickly as possible.* Place the visualization development bag (item 14) upright against a solid support. Pour the vial of brown visualizing agent (item 13) into the bag. *Avoid getting the visualizing agent on the skin, clothing, or in the eyes.* Insert the developed TLC plate, with the spotted end first, as far as it will go into item 14 (Fig. 9). Starting at the bottom, use a straight edge or ruler to squeeze the bag and force the liquid up to, but not over, the opening in the bag. Remove the plate immediately from the bag and hang it upright

until the spots are fully developed (about 30 minutes).

Compare the size and intensity of the spots from the solutions of the standard tablet (7A), sample tablet (7B), and diluted standard tablet (7C). The spots from 7A and 7B should be about the same size and intensity, while the spot from 7B should not be smaller than that from 7C. All spots should line up in a horizontal row and should have moved upward from the place where they were spotted on the plate. Any other spots observed are inactive ingredients or impurities.

• *Step 19.* The sample requires additional analysis if any of the following results are obtained:

—the sample tablet requires 10 times longer to disintegrate than the standard tablet;

—the spot from the sample tablet (7B) is smaller or less intense than that from the diluted standard tablet (7C);

—the main spot from the sample tablet (7B) does not line up horizontally with the spots from 7A and 7C.

Conclusions

The procedure described has been used by a chemist and several undergraduate chemistry students at our laboratory and also corroborated by chemists at other Food and Drug Administration laboratories. All the dose levels shown in Table 1 were assayed by one or more analysts. The method was written using simplified terminology as much as possible to make it readily comprehensible by persons with limited education or laboratory experience.

It should be noted that the method is not intended to simulate the United States Pharmacopeia (USP) tablet disintegration test with respect to the temperature of the medium and the motion of the water and of the tablet in the water, but rather to compare a sample with a standard product having satisfactory characteristics.

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We give special thanks to J.C. Moreira, Instituto Nacional de Controle de Qualidade em Saude (INCQS), Rio de Janeiro, Brazil, for helpful discussions on the experimental approach reported here. We also wish to thank the following collaborators, all of whom are FDA employees, for their contributions to this project: E. Bargo, U. Cieri, A. Clarke, C. Froncheck, A. Hartman, J. Marchin, K. Miller, R. Muzeni, D. Naes, L. Greene Niebruegge, R. Olson, G. Reid, M. Rhodes, M. Robinette, D. Sheffield, and E. Wojtowicz.

Further work on adapting this technique for the analysis of other drugs is being carried out in a cooperative effort between the U.S. Public Health Service and the Institute of Drug Research and Control, Warsaw, Poland, under the direction of Professor W. Wieniawski, Deputy Director.

Résumé

Méthode simple et économique de chromatographie en couche mince pour l'analyse des comprimés de théophylline

L'article décrit une méthode simple et économique de chromatographie en couche mince pour l'analyse de formulations simples de théophylline en comprimés. Aucun appareil ni balance n'est nécessaire et l'analyse peut être réalisée sur le terrain. On a utilisé comme étalon interne des comprimés d'une dose moyenne de théophylline préalablement analysés. Cette méthode permet d'obtenir une précision raisonnable, puisque chaque comprimé est analysé en parallèle avec un comprimé étalon. La précision et l'exactitude sont toutefois inférieures à celles qui peuvent être obtenues dans un laboratoire bien équipé, mais sont tout à fait suffisantes pour une analyse rapide sur le terrain. Cette méthode peut être utile dans les régions rurales ou reculées ne disposant pas d'installations de laboratoire pour le contrôle des médicaments et devrait être applicable également à l'analyse d'autres comprimés ou gélules à principe actif unique.

Reference

1. **Senanayake, U.M. & Wijesekera, R.O.B.** A rapid micro-method for the separation, identification and estimation of the purine bases caffeine, theobromine, and theophylline. *Journal of chromatography*, **32**: 75–86 (1968).